

**REMARKS**

Upon entry of this amendment, claims 13-19 and 26-28 will remain pending, with claims 13, 15 and 17-19 being independent claims.

By the amendment herein, the independent claims have been amended to recite "in an *in vitro* composition, wherein the composition does not include insulin." which language was suggested by the Examiners during an August 24, 2009 telephone interview that will be discussed below.

Entry of this amendment after final rejection and Applicants' filing of a Notice of Appeal is appropriate in that the amendment of the claims is in accordance with the Examiners' suggestion with possible allowance of the application, and is being made in response to a telephone interview which was conducted based upon Applicants' arguments presented in several responses regarding the presence of insulin in the technique disclosed by Engman, which arguments had not been addressed in any of the Final Office Action and the Advisory Actions.

Reconsideration and allowance of the application are respectfully requested.

**Discussion of Telephone Interviews**

Arguments express appreciation for the courtesies extended by Examiner Daniel Sullivan and Examiner Sudhakar Katakam during August 5 and 24, 2009 telephone interviews with Applicants' representative Arnold Turk.

During the August 5, 2009 telephone interview, Applicants' representative repeated arguments as presented in Applicants' responses filed previous to and in response to the Final Office Action. In particular, Applicants' representative indicated that the Final Office Action and the Advisory Actions did not respond to Applicants' argument that Engman includes insulin in his

experiment and was experimenting with ebselen as an inhibitor not a substrate. The Examiners indicated that they would review the record, and that a further interview should be arranged to further discuss the rejection of record and Applicants' claimed subject matter.

During the August 24, 2009 telephone interview, the Examiners noted that Engman used insulin in his assay, and also pointed out that Applicants' specification includes an example wherein the activity of thioredoxin reductase was determined in an insulin assay. The Examiners also noted that Applicants' originally filed application includes examples with and without insulin, and suggested amending the claims to recite that the claimed compositions do not include insulin, which would be in accordance with the originally filed application. Moreover, the Examiners suggested amendment of Applicants' independent claims, and indicated that such amendment should remove the rejection of record.

### **Response To Rejection**

Applicants once again note that the following rejection is set forth in the Final Office Action:

Claims 13-19 and 26-28 are rejected under 35 U.S.C. 102(b) as being anticipated by Engman et al. (hereinafter "Engman"), "Diaryl Chalcogenides as Selective Inhibitors of Thioredoxin Reductase and Potential Antitumor Agents", Anticancer Research, Hellenic Anticancer Institute, Athens, GR, Vol. 17, No. 6D, 1997, pp. 4599-4605.

Applicants also once again submit that the rejection is without appropriate basis in that (as previously argued by Applicants throughout the lengthy prosecution of this application) Engman discloses that ebselen was found to be an inhibitor of human thioredoxin reductase. Engman does not disclose the use of ebselen as a substrate for thioredoxin reductase, and uses excess insulin. See,

for example, the abstract of Engman. Engman merely concludes that ebselen is an inhibitor of thioredoxin reductase. As stated in Engman's abstract, ebselen is not a competitive inhibitor for thioredoxin, but for thioredoxin reductase. Moreover, Engman uses conditions in his experiments apparently based upon his understanding that ebselen is a competitive inhibitor for thioredoxin reductase and not a substrate for thioredoxin reductase. These conditions would not appear to provide the results as asserted in the Final Office Action and in the Advisory Actions, especially in view of the excess amount of insulin included in the experiments.

For the sake of brevity and in view of the indication during the above-noted August 24, 2009 telephone interview that the rejection of record should be withdrawn, Applicants are not repeating each of the arguments as previously set forth in the response, but incorporate those arguments herein as if set forth in their entirety, including the remarks set forth in the Supplemental Reply Under 37 C.F.R. 1.116, filed June 23, 2009. However, Applicants note that **the rejection must establish that under the conditions of the assays disclosed by Engman, including excess insulin, the processes recited by Applicants would be present so that, as required in an anticipation rejection, each and every feature recited in Applicants' claims is present in Engman.**

Therefore, Engman does not teach each and every feature recited in Applicants' claims. Engman does not constitute anticipatory prior art as asserted in the rejection, because:

(1) Engman does not disclose, as recited in Applicants' independent claim 13, a method for reduction of a substrate with thioredoxin reductase, comprising combining the thioredoxin reductase, the substrate and NADPH in an *in vitro* composition, wherein the composition does not include insulin, under conditions to reduce the substrate, the substrate being as recited in Applicants' claim 13.

(2) Engman does not disclose, as recited in Applicants' independent claim 15, a method of enhancing peroxidase activity of thioredoxin reductase, comprising combining NADPH, thioredoxin reductase, thioredoxin and a substrate in an *in vitro* composition, wherein the composition does not include insulin, under conditions to enhance peroxidase activity of thioredoxin reductase, the substrate being as recited in Applicants' claim 15.

(3) Engman does not disclose, as recited in Applicants' independent claim 17, a method of oxidizing reduced thioredoxin by a substrate, the method comprising combining reduced thioredoxin and a substrate in an *in vitro* composition, wherein the composition does not include insulin, under conditions to oxidize the reduced thioredoxin with the substrate, the substrate being as recited in Applicants' claim 17.

(4) Engman does not disclose, as recited in Applicants' independent claim 18, a method for reducing a peroxide comprising combining thioredoxin, thioredoxin reductase, NADPH and a substrate in an *in vitro* composition, wherein the composition does not include insulin, under conditions to reduce the peroxide, the substrate being as recited in Applicants' claim 18.

(5) Engman does not disclose, as recited in Applicants' independent claim 19, a method of preventing peroxidation of a substance comprising combining thioredoxin, thioredoxin reductase and NADPH with a substrate in an *in vitro* composition, wherein the composition does not include insulin, under conditions to prevent peroxidation of the substance, the substrate being as recited in Applicants' claim 19.

The Examiner is reminded that in contrast to the prior art of record, the present invention recognizes and demonstrates that ebselen is a substrate being reduced by NADPH and thioredoxin reductase with a low  $K_m$ -value meaning that it is a very good substrate undergoing unlimited cycles

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of oxidation/reduction in the presence of hydrogen peroxide without affecting the activity of the enzyme. The reduced ebselen is called ebselen selenol and has the Se-N bond broken by reduction. The selenol is oxidized back to ebselen by hydrogen peroxide or another peroxide and a new cycle starts. The reaction is ultimately driven by NADPH. Reduced thioredoxin strongly enhances the thioredoxin reductase reaction which is also proven by determination of the rate of reduction of ebselen by reduced thioredoxin using kinetics with tryptophan fluorescence. The result, never seen before, is that ebselen is a very efficient oxidant of reduced thioredoxin.

Accordingly, for at least the reasons set forth above and in Applicants' previous responses, each of the pending claims is patentable over Engman, and the rejection should be withdrawn.

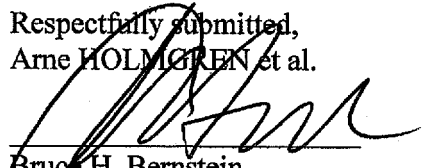
#### CONCLUSION

In view of the foregoing, the Examiner is respectfully requested to reconsider and withdraw the rejection of record, and allow each of the pending claims.

Applicants therefore respectfully request that an early indication of allowance of the application be indicated by the mailing of the Notices of Allowance and Allowability.

Should the Examiner have any questions regarding this application, the Examiner is invited to contact the undersigned at the below-listed telephone number.

Respectfully submitted,  
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